United States Environmental Protection Agency

LABORATORY ANIMAL PROJECT REVIEW

Please note:

1. All information in this LAPR is considered privileged and confidential by the IACUC and regulatory authorities.
2. Approved LAPRs are subject to release to the public under the Freedom of Information Act (FOIA). Do not include proprietary or classified information in the LAPR.
3. An approved LAPR is valid for three years.

LAPR Information

LAPR Title:	**EFFECT OF DEVELOPMENTAL EXPOSURE TO HIGH FAT DIETS AND MATERNAL EXERCISE ON SUSCEPTIBILITY TO OZONE IN RATS**
LAPR Number:	**18-06-002**
Principal Investigator	**Exemption 6**
Author of this Document:	**Exemption 6 /RTP/USEPA/US**
Date Originated:	**04/28/2015**
LAPR Expiration Date:	**06/30/2018**
Agenda Date:	**07/29/2015**
Date Approved:	**08/12/2015**
Date Closed:	**07/06/2018**

APPROVALS

Administrative Information

1. Project Title (no abbreviations, include species):
EFFECT OF DEVELOPMENTAL EXPOSURE TO HIGH FAT DIETS AND MATERNAL EXERCISE ON SUSCEPTIBILITY TO OZONE IN RATS

Is this a continuing study with a previously approved LAPR?
No

2. Programatic Information
a. What Program does this LAPR support? Please provide the Research Program, Project, Task Number and Title.
2.62 Sustainable and Health Communities (no task # available at this time)
b. What is the Quality Assurance Project Plan (QAPP) covering this project?
IRP-NHEERL-RTP/TAD/NB/Exemption/2012-01-r07

3. EPA Principal Investigator/Responsible Employee:

Principal Investigator	*Phone Number*	*Division*	*Mail Drop*
Exemption 6	Exemption 6	TAD	MD

	Lotus Notes Address Exemption 6 Exemption 6 Exemption 6 RTP/USEPA/US	***Branch*** NB	

4. Alternate Contact:

Alternate Contact Exemption 6	***Phone Number*** Exemption 6 ***Lotus Notes Address*** Exemption 6 Exemption 6 Exemption 6 Exemption 6 RTP/USEPA/US	***Division*** TAD ***Branch*** NB	***Mail Drop*** MD

SECTION A - Description of Project

1. Explain the study objective(s) in non-technical language such that it is understandable by non-scientific persons. Explain how the benefits from the knowledge gained from this research outweigh the costs to the animals used in this research. If this is a continuing study from a previous LAPR, briefly justify the continuation. Please spell out all acronyms and abbreviations with their initial use.

Background: One key research mandate from Sustainable and Healthy Communities (SHC) task 2.62 is for researchers in NHEERL to develop experimental paradigms that assess if pre-natal and post-natal exposure to calorically rich diets combined with a sedentary life style increases susceptibility to environmental toxicants such as ozone. In order to prepare a formalized plan to SHC for approval, it is necessary to develop a pilot study to determine if treatment of rats prior to and during pregnancy to a combination of a high fat diet and exercise will alter the physiological development of the offspring as they mature into adulthood and affect their sensitivity to ozone exposure.

Objective of study:

Perform a study that assesses the physiological responses of offspring of rats that were subjected perinatally (i.e., prior to and after parturition) to a high fat diet along with an active vs. sedentary lifestyle. We will order the 40 timed-pregnant rats that will have been treated with a control or high fat diet for approximately 42 days prior to insemination and start them on gestational day 1 (i.e., day of being shipped from Raleigh facility) on an exercise or no exercise treatment while continuing the control or high fat diet treatments. We will allow offspring to develop into adulthood and perform a variety of metabolic and behavioral tests on them to discern if the pre- and postnatal treatments led to detectable changes in the offspring.

We will also expose some of the adult offspring to ozone to evaluate if their perinatal treatments to exercise and/or high fat diets affected their susceptibility to ozone. Ozone is a well known air pollutant that leads to various respiratory symptoms in humans.

It is important to note that we consider this to be a pilot study to explore a variety of complex interactions of perinatal exercise and high fat diets. Nonetheless, we conclude that it is prudent to perform this study with an adequate sample size such that the data can be analyzed statistically and the data could eventually be used for publication. Hence, we are asking for an N of 10 per treatment group in the exposure of the pregnant rats to the experimental treatments.

2. Scientific rationale for proposed animal use.

a. Why is the use of animals necessary?

Contrasting effects of sedentary vs. exercise and treatment with a calorically rich diet is a complex interaction of

stressors that can only be studied in living animals.

b. Justify the species requested:

The Long-Evans rat is an ideal strain of rat to use in this study. We have shown that it develops obesity naturally and the effects can be attenuated with access to a running wheel and augmented with a high fat diet.

3. How was it determined that this study is not unnecessary duplication?

Search in PubMed over the last 35 years shows that no studies of this nature have been published. Search words used in background research: high fat diet, obesity, exercise, developmental effects.

SECTION B - In Vivo Procedures

1. Briefly describe the experimental design. Include descriptions of the age, weight and sex of the animals. Supplementary information may be attached at the end of the LAPR, but please include critical information within the body of the LAPR.

Protocol (for specific assignments and testing of offspring, see attached workbook):

1. There will be 40, 30 day old female rats. LE rats at 30 days of age will be split into two dietary groups; control and high fat diet (60% calories from fat). This will be done at Charles River labs. After 42 days of diet treatment, the rats will be paired with males. The timed-pregnant rats will be shipped from Charles River (CR) in Raleigh on gestational day 1 (i.e., they will be bred the previous night; rats with sperm plugs are shipped the following day to our facility). See attached specification sheets for control and high fat diets. Note that we are requesting 40 rats with the anticipation that some rats may not deliver pups. The worse case would be a 20% failure rate, resulting in an N of 8 per treatment group in the post-natal tests. The maximum N would be 10 if there is 100% pregnancy and delivery success.

2. Upon receipt, the timed pregnant (TP) rats will continue on the control or high fat diet and half the rats from each diet group will be housed in cages with running wheels (upon arrival, all rats housed one per cage). Note that running wheels are considered enrichment. In addition, all cages will be provided with enviro-dri as enrichment as well as nesting material when rats give birth. Beta chip bedding will be used for all animals.

3. Dietary treatments are continued in the exercise and sedentary groups. Body composition will be determined in the pregnant rats one week prior to parturition. Rats are allowed to give birth naturally.

4. Running wheels will be removed from cages on PND 8-10 to avoid any injury to the pups.

5. Litters are culled to 8 (4 M and 4 F) on PND 4-6 and weaned on PND 21. Offspring are pair housed at weaning.

6. After weaning, the dams will be allowed to recover from the exercise and diet treatments for 60 days. During this time, we will monitor body composition every two weeks, looking for recovery of %body fat.

The offspring are evaluated with a variety of tests as explained below in B5c.

2. Justify the number of animals. Include explanation (e.g., biological, statistical, regulatory rationale) for the number of animals needed for each treatment group, and the overall number

requested for the duration of the LAPR.
All tests, including body composition, motron activity chamber (referred to as "motron"), metabolism, glucose tolerance, running wheel performance, and ozone exposures will have an N of 8 to 10. Assuming no failed pregnancies, we will have an N of 10 per treatment group. Meaning one rat from each litter per gender will be taken for a given test. Both males and females will be used in the post-weaning tests. In the majority of the tests to be performed in this LAPR, we have used power calculations to show than an N of 8 is sufficient for statistical analysis of these data. An N of 10 would give us additional statistical power.

For a detailed presentation of the fate of each pup for this study, the reader is referred to the worksheets attached at the end of this document.

3. State how many animals over the study period are expected to be used under the following three categories of pain/distress (USDA nomenclature as defined in the instructions): Please enter numbers only.

Categories	*Adults*	*Offspring*
C) Minimal, transient, or no pain/distress:	40	320
D) Potential pain/distress relieved by appropriate measures:		
E) Unrelieved pain/distress:		

4. Does this LAPR include any of the following:

☐ Restraint (>15 Minutes) ☐ Survival surgery
☐ Food and/or water restriction (>6 Hours) ☐ Non-survival surgery

5. Category C procedures. Describe each procedure separately, include details on the following:

a. Treatments (e.g., dosages, duration of exposure, route, volume, frequency):
Control and high fat diets sold by Harlan (see attachment); 60% calories from fat in high fat diet.

Ozone exposure; Ozone; 0.8 ppm for 5 hrs.

Adult offspring (~90 days of age) will be exposed to 0.8 ppm ozone in Hinner chambers for 5 hr. Then, within one hour after termination of exposure, rats are placed in EMKA plethysmography chambers to measure basic ventilatory parameters. This test takes about 10 min to complete on 8 rats. Rats are then terminated following day. Cellular and protein biomarkers of inflammation are evaluated in lungs.

Details of ozone exposure:

Ozone is generated from oxygen by a silent arc discharge generator (OREC, Phoenix, AZ), and its entry into the Rochester style “Hinners” chambers is controlled by mass flow controllers. The ozone concentrations in the chambers is monitored by photometric ozone analyzers (API Model 400). Air temperature and relative humidity are monitored continuously throughout the study. Ozone had no effect on environmental variables; air temperature and relative humidity in the four chambers is maintained at ~23 °C and 46%, respectively.

The rats are placed in individual stainless steel wire exposure cages (27.3 cm long x 14.6 cm wide x 7.75 cm tall) which were part of a 16 cage unit. Animals are exposed to HEPA-filtered room air (0 ppm) or 0.8 ppm ozone for 5 h/day. Exposure began at 0800 hr. Rats are weighed prior to and after exposure.

b. Survival Blood Collections (method, volume, frequency):

c. Testing methods (including non-stressful dietary restrictions/modifications, mild non-damaging electric shock):
The following tests will be performed on the offspring as the offspring age. Overall, provided

that all dams give birth, a total of 10 rats of each sex and from each of the four exercise/diet groups will be subjected to the following tests. Thus, 80 rats would be subjected to each test:

A. All pups will be examined for endpoints of sexual differentiation Exemption 6 This involves simple handling and visual evaluation of pups prior to weaning.

B. Body composition. Body composition is to be performed with Bruker minispec. We expect the sedentary/high fat diet treatment will lead to indicators of obesity in offspring. At 21 days of age, some offspring will be placed on the high fat diet (one male and one female rat from each litter). Hence, we will study how the perinatal treatment of high fat diet and exercise affects the tendency to develop obesity in offspring that are fed either a control or high fat diet. % fat, lean, and fluid is measured by placing rats in Bruker minispec body composition system. Completely non-invasive test requiring brief restraint for 1 minute. Rats are weighed, placed in Plexiglas tube and the tube is inserted into bore of Bruker system to collect data. That rat is removed and returned to home cage (see attachment for details).

C. Running wheels. Female weaned pups will be placed in cages with running wheels to determine if diet/exercise pre-treatment affects the free-running activity of offspring. The running wheel system consists of a commercially made (Starr Life Sciences) stainless steel, wire wheel (33 cm diameter; 1.02 m circumference) that is positioned in a standard acrylic cage. Wheel revolutions are detected with a magnetic switch positioned near the wheel. The activity of 40 wheels is monitored and analyzed simultaneously. Rats have continuous access to the running wheel that is used primarily during the dark cycle. Food and water is supplied through the top of the cage. Note, we are only assessing this test in females because they run very well on the wheels compared to males.

D. Motron activity chamber. Offspring horizontal and vertical motor activity will be measured for 30 min in the Motron chambers. Chambers consist of illuminated boxes (33×21×26 cm) with photocells assess horizontal and vertical motion. 6 rats can be evaluated in separate chambers at one time. Rats tested at ~60 days of age. We predict that exercise training will lead to an attenuation in motor activity when tested in these chambers.

E. Respiratory data will be collected from adult offspring to assess baseline responses at ~60 days of age and the day after exposure to ozone to assess effects of ozone on ventilatory dysfunction. After ozone exposure, rats will be returned to their home cage transiently and then placed in a plethysmograph chamber to collect respiratory data. A eight chamber whole-body plethysmograph system (using EMKA software) measures respiratory frequency, minute ventilation, tidal volume and the enhanced pause (Penh), an index of airflow limitation and a surrogate for bronchoconstriction. Rats will be housed in the chamber for ~6 min while data are collected and then returned back to their home cage.

F. Metabolism. Metabolic rate (MR) and respiratory quotient (RQ) will be measured in adult offspring at once between 60-90 days of age. These tests will be performed to assess the impact of perinatal exercise and dietary treatment on metabolic rate and contribution of different substrates to energy metabolism. Individual calorimeters measure oxygen consumption, carbon dioxide production, and RQ. RQ is calculated to determine the relative use of substrates (i.e., carbohydrate vs. fat). The calorimeter chambers are made of clear plastic (length 30.4 cm, width 19 cm, height 19 cm) and had perforated plastic floors to allow feces and urine to drop through.

Fresh air (i.e., from the animal room) is first run through a desiccator to remove water and then pumped into each calorimeter chamber at a controlled rate of 2.0 L/min. The calorimeters are housed in the animal vivarium maintained at 22 °C. Food and water will be freely available inside the calorimeters.
On the day of testing, each rat is placed in an individual calorimeter at 9:00 AM and remained undisturbed for the next 22 hours. At the end of the session, the rats are returned to their home cages. All parts of each calorimeter will be washed before starting the next test.

G. Glucose tolerance test (GTT). This is a gold standard test for indications of symptoms related to type 2 diabetes. Rats are fasted for at least 6 hrs. A bolus of glucose (2 g/kg; IP; 10 ml/kg) is injected intraperitoneally. Tail blood is sampled prior to injection and every 30 min after injection for two hours. A tail nick with a sterile needle is made to collect a droplet (1 ul) of blood for glucose measurement.

For each test, baseline blood glucose levels will be measured following 6-8 hours fasting by pricking through the tip of the tail using a sterile 25 gauge needle that was wiped with an alcohol swab and sterile gauze. Approximately 1 ul of blood will be brought into contact with the glucometer strip attached to a Bayer Contour glucometer. Glucose levels are measured within 5 sec and recorded. Once baseline glucose is measured, pharmaceutical grade glucose will then be injected intraperitoneally (maximum of 1.5 g/kg body weight/10 mL) and blood glucose will be measured at 30, 60, 90, and 120 min post-glucose injection. Each rat will have a total of 5 glucose measurements (baseline plus four times following intraperitoneal injections of glucose) during each tolerance test. The dose volume of glucose will be 10 ml/kg body weight. These volumes have been used in published studies for rats. We will use ACS grade glucose (40% concentration) purchased from Sigma Aldrich. The glucose solution will be made fresh each time using new pharmaceutical grade saline vials. Sterile syringes and needles will be used for each rat for intraperitoneal injection.

d. Animal restraint and confinement beyond routine housing and handling. Include a description of the type of restraint device, acclimation to device, duration of restraint:

e. Breeding for experimental purposes (e.g. length of pairing, number of generations):
All breeding will be done at Charles River using their protocols. Offspring generated on site here.

f. Describe how animals will be identified and monitored. Include description of identification procedures. (For example, if transponders are used, how are the animals prepared?) Include frequency of observations and by whom:
All TP females are housed one per cage. Starting on GD 20, rats will be checked frequently during the day for signs of labor/parturition. Any rats in difficult labor will be closely monitored and may be euthanized. Monitoring will be made by Exemption 6 and Exemption 6. Offspring will be pair housed immediately at weaning and are marked periodically for identification with tail marks using approved "Marks-A-Lot" markers.

Rats are checked daily by laboratory staff Exemption 6 and/or by animal care staff

6. Non-surgical Category D or E procedures. Describe each procedure separately, include details on the following (Also fill in Section B.9).

a. Treatments (e.g. dosages, duration of exposure, route, volume, frequency):

b. Blood Collection (Provide a description of the procedure including method, volume, and frequency if appropriate. Indicate if the procedure is survival or terminal. Include preparatory methods, descriptions of incisions, etc.):

c. Testing methods:

d. Restrictions placed on the animals' basic needs (e.g., food and/or water restriction, light cycles, temperature). Provide details regarding the length of restriction. Describe the method(s) for assessing the health and well-being of the animals during restriction. (Amount of food or fluid earned during testing and amount freely given must be recorded and assessed to assure proper nutrition.):

e. Describe how animals will be monitored (e.g., frequency of observations, by whom):
..

f. Analgesia (Category D Procedures) - list drugs, dosages, route of administration and frequency:

g. If treatment-related deaths are expected, this must be thoroughly justified. Death as an endpoint is highly discouraged:

7. Surgical Category D and E procedures. Indicate if the surgery is survival or terminal. Describe each surgical procedure separately, include details on the following (Also fill in Section B.9)

a. Complete description of surgical procedure including presurgical preparation, aseptic technique, surgical closure, etc:

b. Anesthetic regimen (Drugs, dosages, volume, route of administration and delivery schedule). The use of paralytic or neuromuscular blocking agents w/o anesthesia is prohibited:

c. Postoperative care (thermal support, special feeding, responsible personnel, removal of sutures/staples, frequency and duration of monitoring including weekend and holiday care):

d. Post operative analgesics (drugs, dosage, and volume and route of administration, frequency):

e. Will any animal be subject to more than one surgical procedure over the course of its lifetime, either here at NHEERL or elsewhere?

○ Yes ● No

f. Identify any surgical procedures performed at other institutions or by vendors:

8. Humane interventions (for treatments/procedures in all categories).

a. What resultant effects, if any, do the investigators expect to see following procedures or treatment? Please include transitory as well as permanent effects. Examples might include lethargy, ataxia, salivation or tremors. Indicate the expected duration of these effects.

Running wheel exposure should have no negative impact on rats health. We have used them for over a year and rats run many kilometers/hr each night. This level of ozone exposure (0.8 ppm for 5 hr) has been shown to be well tolerated by rats. We expect significant effects on ventilatory parameters measured the day of and one day after ozone exposure. The rats should recover rapidly from this air pollutant.

Pregnant and nursing rats and pups will be closely monitored. Exemption 6 is primarily responsible. She has over 30 years of experience in performing these types of developmental studies.

Animals will not be treated with toxicants. However, if animals show symptoms of physical injury, dystocia, rough hair coat, or deteriorating body condition we will euthanize or otherwise follow AV recommendations.

b. State the criteria for determining temporary or permanent removal of animals from the study. Describe actions to be taken in the event of deleterious effects from procedures or chemical exposures. Describe actions to be taken in the event of clinical health problems not caused by procedures or exposures.

Healthy rats, as are being used in this study, are expected to tolerate this level of ozone exposure with no significant problems. We have used this concentration and time of exposure for many studies over the past 8 years. However, if animals shows signs of labored breathing during exposure, they will be removed from the chamber. They will be closely monitored and allowed to recover; however, if there is evidence of severe distress or physical injury, the rat will be euthanized. Other testing is considered to have no adverse impact on health of rats.

If animals show symptoms of physical injury, rough hair coat, or deteriorating body condition we will euthanize or otherwise follow AV recommendations. If animals show signs of

dystocia, we will euthanize immediately. Signs of dystocia (difficult labor) would include bleeding from vagina, other vaginal discharge, straining without producing any pups.
Other signs of uterine infection may include rough hair coat, dehydration, weight loss, and anorexia or decreasing appetite. If these occur, AV will be notified and case discussed.

9. Alternatives to pain and distress (Category D and E Procedures only). Provide narrative regarding the sources consulted to ascertain whether acceptable alternatives exist for potentially painful/distressful procedures. Include databases searched or other sources consulted, the date of the search and years covered by the search, and key words and/or search strategy used. Assistance with searches is available through the EPA Library Staff.

SECTION C - Animal requirements

Describe the following animal requirements :

1. Indicate the number of animals required over the study period for this protocol. Please enter numbers only.

a. Animals to be purchased from a Vendor for this study:	40
b. Animals to be transferred from another LAPR:	
LAPR Number that is the source of this transfer:	
c. Animals to be transferred from another source:	
d. Offspring produced onsite (used for data collection and/or weaned):	320
e. TOTAL NUMBER of animals for duration of the LAPR	360

2. Species (limited to one per LAPR): Rat(s)

3. Strain: Long Evans Rat(s)
Describe special requirements for animals with altered physiological responses (e.g., genetically altered, aged)

4. Sources of animals:
Charles River

5. Provide room numbers where various procedures will be performed on animals:
Exemption 6 Exemption 6 body composition; EMKA (ventilatory function) Exemption 6 Exemption 6; ozone exposure Exemption 6 Exemption 6; Motron Exemption 6 Exemption 6 Exemption 6; metabolism Exemption 6 Exemption 6

6. Will any animals be housed in areas other than the animal facility longer than 12 hours? If so, state location. Such areas require prior IACUC approval as a satellite facility before LAPR can be reviewed.

Room Numbers:

7. Describe any transportation and containment methods involved in moving animals between EPA buildings, or between EPA and other institutions (excluding any commercial shipments)

8. Describe any unusual housing or husbandry requirements, or acclimation requirements. Justify any treatment beginning less than 3 days after arrival.
We request that pregnant rats be placed in cages with running wheels upon arrival from Charles River. We want to initiate the exercise protocol as soon as possible after fertilization.

It requires several days for rats to acclimate to wheels, meaning that running activity per night increases steadily with each day. The sooner they get started on the wheels, the greater the amount of exercise they will have performed by the time they give birth.

9. Describe special assistance requested of the animal contract staff, including procedures and dosing. NOTE, this request must be submitted separately to the Animal Resources Program Office (ARPO)

10. Housing and Enrichment.
The IACUC encourages the use of environmental enrichment whenever possible (see IACUC website for details). Provide details on how the animals will be housed, including type of cage (e.g., solid bottom or wire screen), bedding material, number of animals per cage, and environmental enrichment. Note that housing rodents individually without environmental enrichment requires justification.
All rats are housed in standard, large shoe box acrylic cages. Note that running wheels are considered enrichment. In addition, all cages are provided with enviro-dri as enrichment as well as nesting material when rats give birth. Beta chip bedding will be used for all animals. Enviro-dri is also provided in cages housing offspring after weaning.

SECTION D - Agents Administered to Animals

1. Identify all hazardous and non-hazardous agents to be administered to living animals. For agents requiring a Health and Safety Research Protocol (HSRP), provide the title of the approved HSRP for each such agent. If no protocol is required for an agent deemed potentially hazardous (e.g. nanoparticles, recombinant DNA), describe the safety precautions to be used.
Provide maximum dosing levels and route-appropriate LD50s (where available) for each agent used for dosing.
Control and high fat diet; free feeding.
Ozone exposure; 0.8 ppm ozone by inhalation for 5 hr.
LC50 for 4 hr for rat reported at 4.8 ppm

2. Describe compounds to be administered to animals.
a. Are all substances pharmaceutical grade? If not, provide a scientific justification for the use of non pharmaceutical grade compounds.
Glucose is pharmaceutical grade. The glucose solution will be made fresh each time using new pharmaceutical grade saline vials.
Ozone is generated on site. Pharmaceutical grade does not apply to ozone.
b. Describe any plans to administer human or animal tissues, blood or body fluids to the animals in the LAPR. Provide information to assure that such material is pathogen free. Indicate what safety precautions are necessary for handling the material.

c. Provide a statement regarding any safety precautions necessary for handling any of these materials.

NOTE: Any unresolved health/safety questions which arise during IACUC review of this LAPR will require consultation with the Safety, Health, and Environmental Management Office.

SECTION E - Personnel Training and Experience

1. Identify all project personnel conducting animal experimentation. Specify the techniques for which they have responsibility, and their relevant training and experience. Additional personnel may be added to the table below as a group (by Division) for Category C procedures. By so doing you are

giving assurance that these personnel have received all required training and are qualified to perform the Category C techniques requested.

Use this area to type in additional personnel information not available in the table drop-down lists:

Hint: *The names in the first 2 lines of the table below are filled automatically from the Principal Investigator & Alternate Contact fields. A new line will be made available when a name is selected & upon leaving the name field (i.e. tabbing or clicking in another field).*

NAME	ROLE	SPECIFIC RESPONSIBILITY	RELEVANT TRAINING
Exemption 6 Exemption 6 Exemption 6	Principal Investigator	study design	40 years of animal research experience. Received all required NHEERL training
Exemption 6	Technical Staff	body composition, EMKA, ozone exposure, husbandry of dams/pups. Reproductive assessment.	40 years of animal research experience. Received all required NHEERL training
Exemption 6 , Exemption 6 Exemption 6	Technical Staff	body composition, EMKA, ozone exposure	10 years of animal experience. Received all required NHEERL training
Exemption 6	Associate Principal Investigator	reproductive assessment	20 years experience. Received all required NHEERL training
Exemption 6	Technical Staff	data analysis	20 years experience. Received all required NHEERL training.
RTP-NHEERL	Tech Support	Category C Procedures	All NHEERL required training is complete.

SECTION F - Animal Breeding Colonies

This section pertains to the breeding of animals for maintenance of ongoing animal colonies. Do not include breeding that is part of experimentation and accountable under Section C.

Describe:

1. Estimated number of breeding pairs and liveborn per year

2. Breeding protocols and recordkeeping

3. Methods for monitoring genetic stability

4. Disposition of all offspring and retired breeders that are not used in accordance with the procedures described in this LAPR

SECTION G - Euthanasia

1. When will the animals be euthanized relative to experimental procedures?

At culling, all pups not needed will be euthanized by decapitation before PND 7 using scissors. Scissors will always be pre-sharpened. Many pairs will be available. Exemption 6 will be performing this procedure.

Dams will be euthanized approximately two months after parturition. Offspring will be euthanized following ozone exposure. Non-ozone exposed offspring will be monitored for changes in body composition as treatments with running wheels and high fat diets progress. Euthanized no later than 1 year of age.

2. Describe the euthanasia techniques:

Method(s): Anesthesia plus vital organ transection
Agent(s): carbon dioxide
Dose (mg/kg): 100%
Volume: to effect
Route: inhalation

Source(s) of information used to select the above agents/methods:

2013 AVMA Guidelines on Euthanasia.

3. Provide justification and references for any euthanasia agent or method that is not consistent with recommendations of the American Veterinary Medical Association (AVMA) Guidelines for Euthanasia (e.g., cervical dislocation or decapitation without anesthesia; cervical dislocation in rodents weighing more than 200 grams).

4. Describe how death is to be confirmed.

Vital organ section

SECTION H - Disposition of Used and Unused Animals

Describe the disposition of any animals remaining after project completion.

The IACUC encourages investigators to reduce the overall number of animals used at NHEERL. Would you consider transferring any unused animals from this LAPR to another approved LAPR?

● Yes ○ No

SECTION I - Assurances

1. Animals will not be used in any manner beyond that described in this application without first obtaining formal approval of the IACUC.
2. All individuals involved in this project have access to this application, are aware of all EPA policies on animal care and use, and are appropriately trained and qualified to perform the techniques described.
3. Thorough consideration of the three "R"'s (Replacement, Reduction, Refinement) has been given, as applicable, to a. the use of animals, and b. procedures causing pain or distress (with or without analgesia/anesthesia), including death as an endpoint. The minimum number of animals required to obtain valid experimental results will be used.
4. The Attending Veterinarian has been consulted in regard to any planned experimentation involving pain or distress to animals.
5. The IACUC and Attending Veterinarian will be promptly notified of any unexpected study results that impact the animals' well-being, including morbidity, mortality and any occurrences of clinical symptoms which may cause pain or indicate distress.
6. All procedures involving hazardous agents will be conducted in accordance with practices approved by the Safety, Health, and Environmental Management Office.
7. I certify that I am familiar with and will comply with all pertinent institutional, state and federal rules and policies.

8. The IACUC has oversight responsibilities for animal care and use, and may request consultation or feedback regarding the conduct of in vivo procedures, progress and accomplishments, and any problems encountered.

EPA Principal Investigator	Certification Signature Date
Exemption 6 Exemption 6	04/29/2015

Submitted: 06/24/2015

Certification:

Certification by EPA Supervisor (Branch Chief or Division Director) that the project described herein has been reviewed and approved on the basis of scientific merit:

Branch Chief/Division Director	*Approval Date*	*Phone Number*	*Division*	*Mail Drop*
Exemption 6	06/24/2015 by Exemption 6 Exemption 6 Exemption 6 RTP/USEPA/US	Exemption 6 ***Lotus Notes Address*** Exemption 6 Exemption 6 Exemption 6 RTP/USEPA/US	TAD ***Branch*** DTB	MD ***Submitted to Branch Chief for Approval*** 06/24/2015 11:41 AM

ATTACHMENTS

LAPR 18-06-002 DR approval.pdf
LAPR 18-06-002 PI Resp 3.pdf
LAPR 18-06-002 PI Resp 2.pdf
LAPR 18-06-002 PI Resp.pdf
Experimental design pictorial_high fat diet and exercise during pregnancy.pptx
Exemption 6 Exemption 6 Exemption 6 up plan - PMP.xlsx
Harlan TD.06414 high fat.pdf
Harlan TD.08806 control for HF.pdf
OPERATION OF BRUKER FAT ANALYZER_October 20 2010.doc

Actions

First Update notification sent: 05/02/2016
Second Update notification sent:
First 2nd Annual notification sent: 05/02/2017
Second 2nd Annual notification sent: 06/02/2017
1st Expiration notification sent: 04/26/2018
2nd Expiration notification sent: 05/29/2018

History Log: